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Age-Dependent Effects of 6-Hydroxydopamine on Locomotor Activity in the Rat

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16. ABSTRACT

This experiment examined the effects on locomotor activity of intraventricular 6-hydroxydopamine (6-OHDA) administered to developing and adult rats. 6-OHDA was administered subsequent to pargyline treatment at 3 and 6 days of age; or 6-OHDA was administered subsequent to desmethylimipramine (DMI) treatment (6-OHDA/DMI) at 3 and 6 days of age, 11 and 14 days of age, 20 and 23 days of age, or 46 and 48 days of age. Locomotor activity of vehicle-treated rats assessed in stabilimeter cages peaked between 14 and 16 days of age and subsequently declined to levels characteristic of the adult. Treatment with pargyline and 6-OHDA at 3 days of age, or 6-OHDA/DMI at 3 and 6 or 11 and 14 days of age, did not alter the early rise in locomotor activity but prevented the decline in activity normally seen during the third and fourth weeks of life. When tested as adults, locomotor activity was greater in rats that had been treated with 6-OHDA/DMI at 3 and 6 and at 11 and 14 days of age than in those that had been treated at 20 and 23 days of age. Tretment with 6-OHDA/DMI at 46 and 48 days of age was without significant effect on locomotor activity.

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AGE-DEPENDENT EFFECTS OF 6-HYDROXYDOPAMINE ON LOCOMOTOR ACTIVITY IN THE RAT

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SUMMARY

This experiment examined the effects on locomotor activity of intraventricular 6-hydroxydopamine (6-OHDA) administered to developing and adult rats. 6-OHDA was administered subsequent to pargyline treatment at 3 and 6 days of age; or 6-OHDA was administered subsequent to desmethylimipramine (DMI) treatment (6-OHDA/DMI) at 3 and 6 days of age, 11 and 14 days of age, 20 and 23 days of age, or 46 and 48 days of age. Locomotor activity of vehicle-treated rats assessed in stabilimeter cages peaked between 14 and 16 days of age and subsequently declined to levels characteristic of the adult. Treatment with pargyline and 6-OHDA at 3 days of age, or 6-OHDA/DMI at 3 and 6 or 11 and 14 days of age, did not alter the early rise in locomotor activity but prevented the decline in activity normally seen during the third and fourth weeks of life. When tested as adults, locomotor activity was greater in rats that had been treated with 6-OHDA/DMI at 3 and 6 and at 11 and 14 days of age than in those that had been treated at 20 and 23 days of age. Treatment with 6-OHDA/DMI at 46 and 48 days of age was without significant effect on locomotor activity. 6-OHDA (with pargyline pretreatment) produced large decreases in NE content in telencephalon and diencephalon and in dopamine (DA) content in striatum. 6-OHDA/DMI also produced large decreases in DA content in striatum and, in some of the treatment groups, only small decreases in norepinephrine (NE) content in telencephalon, diencephalon, and brain stem. These data suggest that the maturation of neuronal systems utilizing dopamine as a neurotransmitter is essential for the suppression of locomotor activity normally seen during development. The data further

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suggest that dopamine depletion per se does not lead to increased locomotor activity, but rather it is the destruction of dopamine-containing fibers prior to the normal period of locomotor suppression that increases locomotor activity.

INTRODUCTION

The development of locomotor activity in the rat has been studied extensively[1,3]. Although there are differences in the patterns due to the variety of techniques used to measure locomotor activity, a general pattern of locomotor development has emerged[3,5,10,12,14,18,20]. Activity levels are low in rats for the first two weeks of life. A sharp increase in activity is seen beginning at 10 days and reaches an activity peak at 14 and 16 days of age, which is then followed by a decline in activity in subsequent days to approach lower adult levels by 25–27 days of age. The increase in locomotor activity corresponds with a period of rapid increase in brain catecholamines and the enzymes necessary for their biosynthesis[4,7,13]. Pharmacological studies suggest that the development of brain stem catecholaminergic systems mediates the rise in locomotion[5,6]. The maturation of forebrain cholinergic, serotonergic and catecholaminergic systems has been implicated in the decline in motor activity[5,8,9,17,18]. In the present study, the effect of 6-hydroxydopamine (6-OHDA)-induced catecholamine depletion on the development of locomotor activity was measured in stabilimeter cages. The age at which the animals were treated with 6-OHDA was varied to determine whether effects on locomotor activity were dependent on catecholamine depletion occurring at particular points in development. It was found that rats treated with 6-OHDA before 22 days of age showed increased locomotor activity compared to control rats. The differences in the 6-OHDA-treated rats and the controls were exhibited only after the peaks in locomotor activity seen at 14–16 days of age. In addition, the differences did not occur if the rats were treated with 6-OHDA after 45 days of age.

These findings suggest that catecholaminergic systems are necessary for the normal development of locomotor activity in the rat and that the increased locomotion following administration of 6-OHDA is critically dependent on the stage of development of the catecholaminergic systems.

MATERIALS AND METHODS

Animals

Female, sperm-positive Sprague–Dawley derived rats (Holtzman Company, Madison, Wisc.) were housed individually one week before parturition in temperature-controlled (23 ± 1 °C) colony rooms in which artificial lighting was provided from 06.00 to 18.00 h. Food and water were available ad libitum. Births were recorded to within 12 h. Pups were left undisturbed until 3 days of age, at which time the females were discarded and the males were randomly assigned to litters of 8. Litters were housed in large plastic colony cages. Rats were weaned at 28 days. For the experiments in which 6-OHDA and desmethylimipramine (DMI) were administered on days 46

and 48, male rats were obtained at 30 days of age from the Holtzman Company. These animals were housed 8 per cage in large plastic colony cages and had access to food and water ad libitum.

Apparatus

Locomotor activity was measured in stabilimeter cages modeled after those described by Moorcroft et al.[11]. Up to 30 days of age, rats were tested in cages which measured 20.5 cm long, 10 cm wide and 10.5 cm high and were constructed of 0.63 cm mesh galvanized hardware cloth. Each cage rested on a central fulcrum and an excursion from one side to the other resulted in a 4 mm displacement of the cage which activated a microswitch registering one activity count. After 42 days of age, rats were tested in cages which measured 38 cm long, 18 cm wide and 20 cm high. Each cage rested on a central fulcrum and an excursion from one side to the other resulted in a 6 mm displacement of the cage. Each cage was enclosed in an insulated metal chamber equipped with two houselights (GE 1819, 1.1 W) and a blower that provided ventilation and masking noise. Cages were interfaced with a PDP/8E computer programmed to control session duration and to record activity from each cage at 5-min intervals.

Drugs

6-Hydroxydopamine (6-OHDA) hydrobromide was obtained from Regis Chemical Company, Chicago, Ill. All doses of 6-OHDA are expressed as free base. 6-OHDA was dissolved for injection in a vehicle of 0.9% saline containing 0.1% ascorbic acid. Pargyline hydrochloride was kindly supplied by Abbott Laboratories, North Chicago, Ill. Desmethylimipramine (DMI) was kindly supplied by Lakeside Laboratories, Milwaukee, Wisc.

Experimental procedures

6-OHDA in rats pretreated with pargyline. At 3 days of age, male pups from 3 litters were treated s.c. with pargyline HCl, 40 mg of the salt/kg body weight. One hour later the pups were anesthetized with ether and those from two of the litters were treated with 100 μg 6-OHDA administered intraventricularly. Fifty μg was injected in a volume of 5 μl into each of the lateral ventricles. Pups from the third litter received intraventricular injections of the 6-OHDA vehicle (0.9% saline containing 0.1% ascorbic acid). Several hours after treatment, each litter was randomly assigned to a lactating dam. Locomotor activity was measured for 55-min periods each day from days 10–27. Rats in these experiments were decapitated at 68 days of age and the brains rapidly removed and dissected into the following regions: brain stem, consisting of pons-medulla and midbrain with cerebellum removed; diencephalon; striatum; and telencephalon minus striatum. Samples were frozen in liquid nitrogen until subsequent catecholamine analysis. Dopamine and norepinephrine were determined by the method of Anton and Sayre[2]. Tyrosine hydroxylase activity was measured by the method of Waymire et al.[22], and DOPA decarboxylase activity was measured as described by Porcher and Heller[13].

6-OHDA in rats pretreated with DMI. Twelve litters of 8 males each were used in this study. The 12 litters were randomly assigned, two litters to a group as follows:

Treatments on days 3 and 6. On day 3, pups were injected s.c. with DMI (20 mg/kg) followed 1 h later by the injection of 100 μg in a volume of 10 μl into the right lateral ventricle. This procedure was repeated on day 6 except that the 6-OHDA injection was made into the left lateral ventricle. On days 3 and 6, control rats were injected s.c. with DMI (20 mg/kg) followed 1 h later by the intraventricular injection of 10 μl of vehicle.

Treatments on days 11 and 14. On days 11 and 14, rats were injected with DMI (20 mg/kg) s.c. 1 h prior to 6-OHDA administration. On day 11, rats received 125 μg 6-OHDA injected into the right lateral ventricle in a volume of 10 μl. On day 14, 150 μg of 6-OHDA in a volume of 10 μl was injected into the left lateral ventricle. On days 11 and 14, control rats were injected s.c. with DMI (20 mg/kg) followed 1 h later by the intraventricular injection of 10 μl of vehicle.

Treatments on days 20 and 23. On day 20, DMI (20 mg/kg) was injected s.c. 1 h prior to the intraventricular injection of 150 μg 6-OHDA into the right lateral ventricle. On day 23, 200 μg of 6-OHDA was injected into the left lateral ventricle. Control rats were treated similarly to 6-OHDA/DMI rats on days 20 and 23 except that vehicle was substituted for 6-OHDA in the intraventricular injection.

Measurement of locomotor activity

Rats were numbered and 4 from each litter were tested for locomotor activity. The same animals were utilized for all testing sessions. Activity was measured in the small stabilimeter cages for 1 h every third day from days 10–30. Beginning at 42 days of age, activity was measured in the large stabilimeter cages for 1 h every third day. Rats were killed at 63 days of age and their brains dissected for catecholamine determination as described above.

Treatments on days 46 and 48. Rats were obtained from the supplier at 30 days of age and allowed to acclimate for 10 days before activity testing began. Activity was measured daily for 1 h in large stabilimeter cages on days 40–59. On days 46 and 48, rats were injected i.p. with DMI (20 mg/kg) followed 1 h later by the intraventricular injection of 300 μg of 6-OHDA in a volume of 25 μl. Control rats (46,48) were treated with DMI but vehicle was substituted for 6-OHDA. On day 59, animals were killed and their brains dissected for catecholamine analysis as described above.

Statistical analysis

Biochemical data were analyzed using Student's two-tailed *t*-test. Locomotor activity data were analyzed using the Wilcoxon test for two samples. The differences were considered statistically significant only when the P values were < 0.05.

RESULTS

The development of locomotor activity was similar in vehicle and 6-OHDA-treated (pargyline-pretreated) rats during the phase of rising activity between 10 and

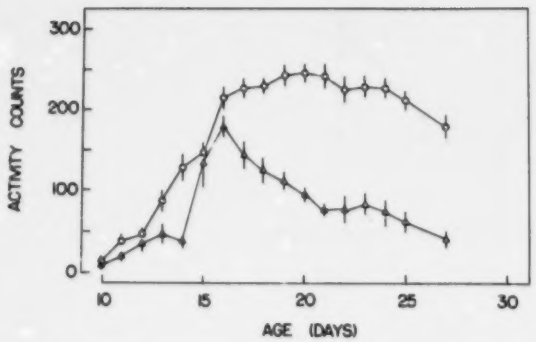

Fig. 1. Locomotor activity of 6-OHDA/pargyline-treated rats compared to vehicle-treated rats. Activity represents counts per 55 min. Differences are statistically significant by Wilcoxon test for two samples from days 17–27. $P < 0.01$. O = 6-OHDA/pargyline, n = 15; △ = vehicle, n = 7. The bars represent the standard error of the mean.

16 days of age (Fig. 1). The activity of 6-OHDA-treated rats diverged markedly from that of vehicle-treated rats over the next 10 days. The locomotor activity of 6-OHDA-treated rats remained elevated while vehicle-treated rats showed the decrease in activity which normally occurs with development[5]. The weights of 6-OHDA and vehicle-treated rats did not differ at any point during the course of this study.

Administration of 6-OHDA (100 μg) to 3-day-old rat pups following pargyline (40 mg/kg) treatment produced substantial decreases in the norepinephrine (NE) content of the diencephalon (—69%) and telencephalon (—97%), but did not significantly affect brain stem NE levels (Table I). Striatal dopamine (DA) content was also lowered (—75%), as were the activities of the biosynthetic enzymes tyrosine hydroxylase (—90%) and DOPA decarboxylase (—75%).

TABLE I

Effect of neonatal treatment with 6-OHDA after pargyline on regional catecholamine and enzyme levels

Values represent the mean ± S.E.M. Norepinephrine (NE) and dopamine (DA) values are expressed as μg/g of tissue. DOPA decarboxylase activity (DD) is expressed as μmoles of product formed/g/h. Tyrosine hydroxylase activity is expressed as nmoles of product formed/g/h.

	Brain stem NE	*Diencephalon NE*	*Telencephalon NE*	*Striatum*		
				DA	*TH*	*DD*
Control (n = 7)	0.62 ± 0.02	0.88 ± 0.03	0.30 ± 0.002	4.31 ± 0.22	3.48 ± 0.20	7.97 ± 0.58
6-OHDA (n = 15)	0.75 ± 0.09	0.27 ± 0.03	0.01 ± 0.003	1.09 ± 0.36	0.36 ± 0.06	2.01 ± 0.18
% Difference	+21	—69*	—97*	—75*	—90*	—75*

* $P < 0.001$.

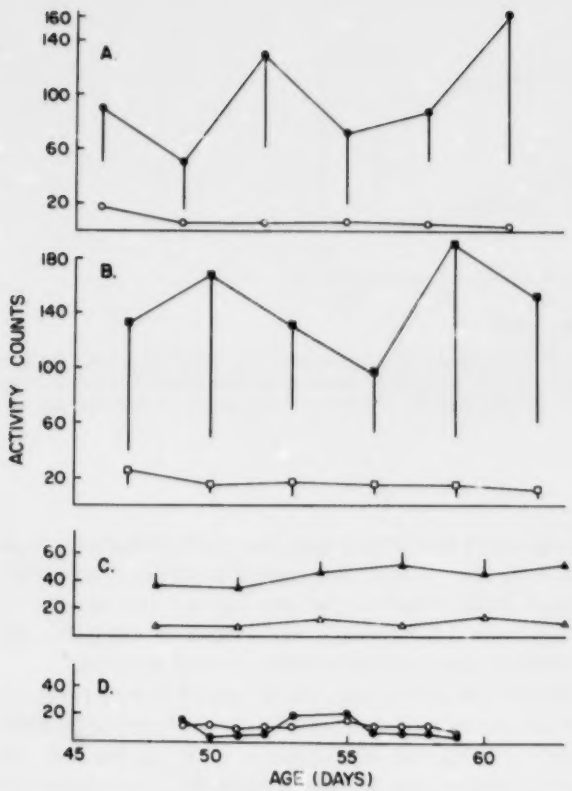

Fig. 2. Effect of age of treatment with DMI/6-OHDA on locomotor activity. A: ● = 6-OHDA/DMI 3,6; O = vehicle 3,6. B: ■ = 6-OHDA/DMI 11,14; □ = vehicle 11,14. C: □ = 6-OHDA/DMI 20,23; △ = vehicle 20,23. D: ● = 6-OHDA/DMI 46,48; ○ = vehicle 46,48. A: 6-OHDA/DMI significantly different from control on days 46, 52, 58, 61 $P < 0.05$ Wilcoxon. B: 6-OHDA/DMI significantly different from control on days 47, 50, 53, 56, 62 $P < 0.05$ Wilcoxon. C: 6-OHDA/DMI significantly different from control on days 48, 63 $P < 0.05$ Wilcoxon. D: 6-OHDA/DMI not significantly different from control 49–59 $P > 0.05$ Wilcoxon. The bars represent the standard error of the mean.

Fig. 2 shows the activity of rats pretreated with DMI followed by 6-OHDA at 3 and 6 days of age, 11 and 14 days of age, 20 and 23 days of age, and 46 and 48 days of age. This locomotor activity was recorded when they were 6–9 weeks old and tested in the large stabilimeter cages. The rats treated as juveniles (days 3 and 6, 11 and 14, or 20 and 23) have significantly higher locomotor activity than the vehicle-treated rats. However, rats treated at 46 and 48 days of age did not exhibit increased locomotor activity. While all the rats treated as juveniles exhibited an increased activity relative to control, those treated within the first two weeks of life displayed higher activity levels than those treated at 20 and 23 days of age.

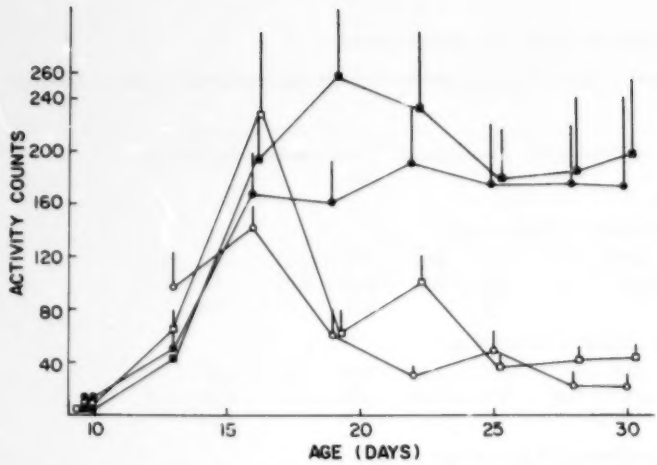

Fig. 3. Failure to develop suppression of locomotor activity produced by DMI/6-OHDA administered within the first two weeks of life. ● = 6-OHDA/DMI 3, 6; O = vehicle 3, 6; ■ = 6-OHDA/DMI 11, 14; □ = vehicle 11, 14; 6-OHDA/DMI 3, 6 significantly different from vehicle days 19–30, $P < 0.05$; 6-OHDA/DMI 11, 14 significantly different from vehicle days 19–30, $P < 0.05$. The bars represent the standard error of the mean.

The early development of locomotor activity in animals treated with DMI followed by 6-OHDA on days 3 and 6 or days 11 and 14 showed a pattern of activity development (Fig. 3) quite similar to that seen in animals treated with 6-OHDA and pargyline. Locomotor activity of both 6-OHDA/DMI pretreated groups remained elevated as had that of the 6-OHDA–pargyline-pretreated group. In addition, treatment of rats at 20 and 23 days of age with 6-OHDA/DMI prevented the normal decline in activity seen by 27 days of life (Fig. 4).

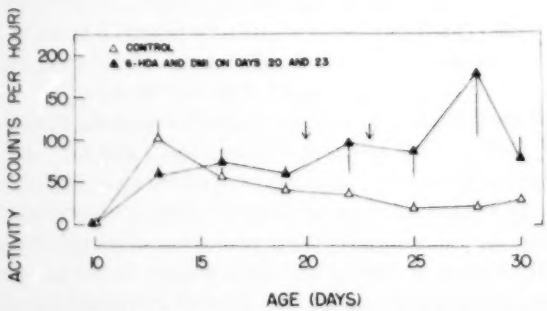

Fig. 4. DMI/6-OHDA treatment at 20 and 23 days prevents the suppression of locomotor activity. ▲ = 6-OHDA/DMI 20, 23; △ = vehicle 20, 23; 6-OHDA/DMI significantly different from vehicle on day 28, $P < 0.05$. The bars represent the standard error of the mean.

TABLE II

Effect of 6-OHDA and DMI on regional catecholamine content

Values represent the mean ± S.E.M. Norepinephrine (NE) and dopamine (DA) values are expressed as μg/g.

	Brain stem NE	*Diencephalon NE*	*Telencephalon NE*	*Striatum DA*
Treatment at 3, 6 days: (killed at 63 days of age)				
Vehicle/DMI (n = 8)	0.54 ± 0.07	0.76 ± 0.09	0.34 ± 0.02	3.31 ± 0.35
6-OHDA/DMI (n = 8)	0.54 ± 0.07	0.82 ± 0.19	0.32 ± 0.01	0.35 ± 0.12
% Difference	0	+8	—6	—89*
Treatment at 11, 14 days: (killed at 63 days of age)				
Vehicle/DMI (n = 8)	0.59 ± 0.07	0.92 ± 0.20	0.30 ± 0.01	4.06 ± 0.23
6-OHDA/DMI (n = 8)	0.49 ± 0.06	0.70 ± 0.10	0.28 ± 0.02	1.24 ± 0.32
% Difference	—17***	—24***	—7	—69*
Treatment at 20, 23 days: (killed at 63 days of age)				
Vehicle/DMI (n = 7)	0.56 ± 0.02	0.78 ± 0.03	0.33 ± 0.02	3.04 ± 0.45
6-OHDA/DMI (n = 6)	0.46 ± 0.02	0.66 ± 0.09	0.29 ± 0.02	0.90 ± 0.25
% Difference	—18**	—15	—12	—70**
Treatment at 46, 48 days: (killed at 59 days of age)				
Vehicle/DMI (n = 7)	0.55 ± 0.02	0.71 ± 0.05	0.28 ± 0.02	2.96 ± 0.19
6-OHDA/DMI (n = 13)	0.35 ± 0.03	0.51 ± 0.06	0.18 ± 0.01	0.50 ± 0.08
% Difference	—36*	—28***	—36*	—83*

* $P < 0.001$; ** $P < 0.01$; *** $P < 0.05$.

In contrast to the 6-OHDA and pargyline treatment, all rats treated with 6-OHDA/DMI showed retarded weight gain relative to control following treatment but this difference lessened with time. At the time of sacrifice, the difference in weight between 6-OHDA/DMI and DMI–vehicle rats was —9% for the groups treated at 3 and 6 days of age, —10% for the groups treated at 11 and 14 days, +3% for groups treated at 20 and 23 days, and —18% for groups treated at 46 and 48 days.

Treatment with 6-OHDA/DMI at each of the 4 ages produced large decrements in striatal dopamine content: —89% in the group treated on days 3 and 6, —69% from the group treated on days 11 and 14, —70% in the group treated on days 20 and 23, and —83% from the group treated on days 46 and 48. DMI administration followed by 6-OHDA was fairly selective for dopaminergic neurons although there were small decreases in the NE content of some of the brain regions in the various treatment groups (Table II). The increase in motor activity for each individual animal was not significantly correlated with the degree of depletion of dopamine in the striatum.

DISCUSSION

These experiments have shown that neonatal administration of 6-OHDA results in a reproducible and long-lasting elevation in locomotor activity. These results confirm and extend the studies of Shaywitz et al.[17,18]. The role of catecholamines in the control of locomotor activity is complex. In the study reported here, long-lasting increases in locomotor activity were recorded in a stabilimeter apparatus from rats who had been depleted of catecholamines as neonates. Shaywitz et al. demonstrated hyperactivity related to central DA depletion which was no longer seen by 4 weeks of age using an observation and sampling method for recording activity[16,18]. In contrast, Smith et al.[21] found that rats depleted of DA and NE, or selectively depleted of DA, show decreased locomotor activity measured as adults while animals in which NE was preferentially reduced showed greater activity than control animals. Smith et al. used circular photocell activity cages to measure the locomotor activity of adults. The different procedures used for measuring locomotor activity as well as the differing routes of 6-OHDA administration (intraventricular in the study reported here, intracisternal in the studies of Shaywitz et al. and Smith et al.) may account for the divergent results.

In the present study, the removal of DA neurons prior to the normal period o developmental suppression of locomotor activity was shown to result in a long-lasting hyperactivity which was maintained throughout the period of observation of 9 weeks. Moreover, the effect appears to be a permanent one and has been observed in rats tested for as long as one year following treatment (McGuire et al., in preparation).

The age at which the animal sustained destruction of dopaminergic fibers was critically important to the resultant effect on locomotor activity. The destruction of DA fibers at either 3 and 6 or 11 and 14 days of age sets activity at a higher level than does a similar degree of DA depletion produced at 20–23 days of age. Strikingly, similar depletions sustained at 46 and 48 days of age result in no measurable effect on locomotor activity. Striatal dopamine was measured in this study and given the relationship between the extrapyramidal system and motor function, it is tempting to conclude that prevention of the maturation of the nigrostriatal dopamine systems is reponsible for the hyperactivity. However, it must be pointed out that dopamine is found in other areas of the brain including the cerebral cortex, the olfactory system and the hypothalamic infundibular system. Since 6-OHDA may well have depleted dopamine in any of these regions, one cannot exclude the possibility that dopamine neurons outside of the striatum may play a role in this developmental effect.

The reduction in telencephalic NE content does not seem to account for the lack of effect on motor activity seen in the rats lesioned at 46–48 days of age, since the decrease in telencephalic NE is of relatively small magnitude and increased locomotor activity was seen in animals with much larger reductions in telencephalic NE following neonatal treatment with pargyline and 6-OHDA. In the present study, NE levels in brain stem were not significantly affected by neonatal 6-OHDA treatment. Other investigators[15,19] have reported increases in brain stem NE from 50 to 100% following subcutaneous 6-OHDA administration on 3 separate occasions. In the present study,

6-OHDA was injected intraventricularly on two occasions. The difference in the total amount of 6-OHDA and the route of administration may account for the differences. Similar results to those presented here have been reported using similar routes of administration and dose[21].

Treatment with 6-OHDA/DMI resulted in decrements in weight gain in all treated rats regardless of the age of treatment. Yet the effects on locomotor activity varied depending on the age of treatment. In addition, rats treated with pargyline and 6-OHDA showed no effect on growth but still demonstrated increased locomotor activity. It is therefore apparent that the weight changes seen in the 6-OHDA/DMI rats probably are not responsible for the effects on locomotor activity.

It has been proposed that forebrain cholinergic or serotonergic mechanisms play a role in the suppression of locomotor activity[5]. The data presented here, along with those of Shaywitz et al.[17,18], suggest that dopaminergic fibers are essential for the suppression of locomotor activity. Furthermore, we have demonstrated that DA depletion per se does not lead to increased locomotor activity, but rather it is the removal of dopaminergic fibers before the normal period of locomotor suppression which results in elevated locomotor activity. The fact that there is a restricted time period during which removal of the dopaminergic system leads to hyperactivity demonstrates that the role of a neurotransmitter system in regulating behavior may be a function of the developmental stage of that system.

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